

The Threshold Approach for Acute Fish Toxicity Testing

GENERAL CONSIDERATIONS

1. In the interest of animal welfare and efficient use of resources, it is important to avoid the unnecessary use of animals whenever possible. In the field of aquatic toxicology, this especially applies to the acute toxicity testing of fish according to OECD TG 203. The threshold approach described hereafter addresses fish toxicity by initially using a single-concentration test (limit test) requiring less fish compared to the full acute fish toxicity study. The selection of a single concentration is based on the derivation of a *threshold concentration* (TC) from reliable algae and acute invertebrate (e.g. daphnia) toxicity data. Fish toxicity is then tested at the TC to consider if fish are more or less sensitive than groups/species for which an E/LC₅₀ is available. If no mortality occurs in the limit test using the TC, the TC might be used as a surrogate LC₅₀ value in the further hazard or risk assessment.
2. The threshold approach proposes both best practice and an ethical benchmark for *in vivo* testing for acute fish toxicity. The threshold approach is based on the observation that fish is not always the most sensitive test species (1, 2). The concept initially described for pharmaceuticals (2) was further developed for chemical substances at the European Commission's Joint Research Centre (3) taking into consideration the requirements of the limit test in OECD TG 203 (4, 5)¹. In addition, several publications confirm the potential of the threshold approach in reducing the number of fish for acute toxicity testing (6, 7), also when applied to other substances than chemicals.
3. The threshold approach is not applicable where a concentration-response relationship and an LC₅₀ derivation are required.

DESCRIPTION OF THE THRESHOLD APPROACH

4. When acute fish toxicity data need to be generated, this guidance document recommends that the threshold approach be applied whenever possible. The whole approach might include the performance of tests in a step-wise manner according to the following OECD Guidelines:

- TG 201 – Freshwater Alga and Cyanobacteria, Growth Inhibition Test
- TG 202 – Daphnia sp. Acute Immobilisation Test
- TG 203 – Fish, Acute Toxicity Test (Limit test, paragraph 20)
- TG 203 – Fish, Acute Toxicity Test.

It is recommended that the following step-wise procedure be utilized (Figure):

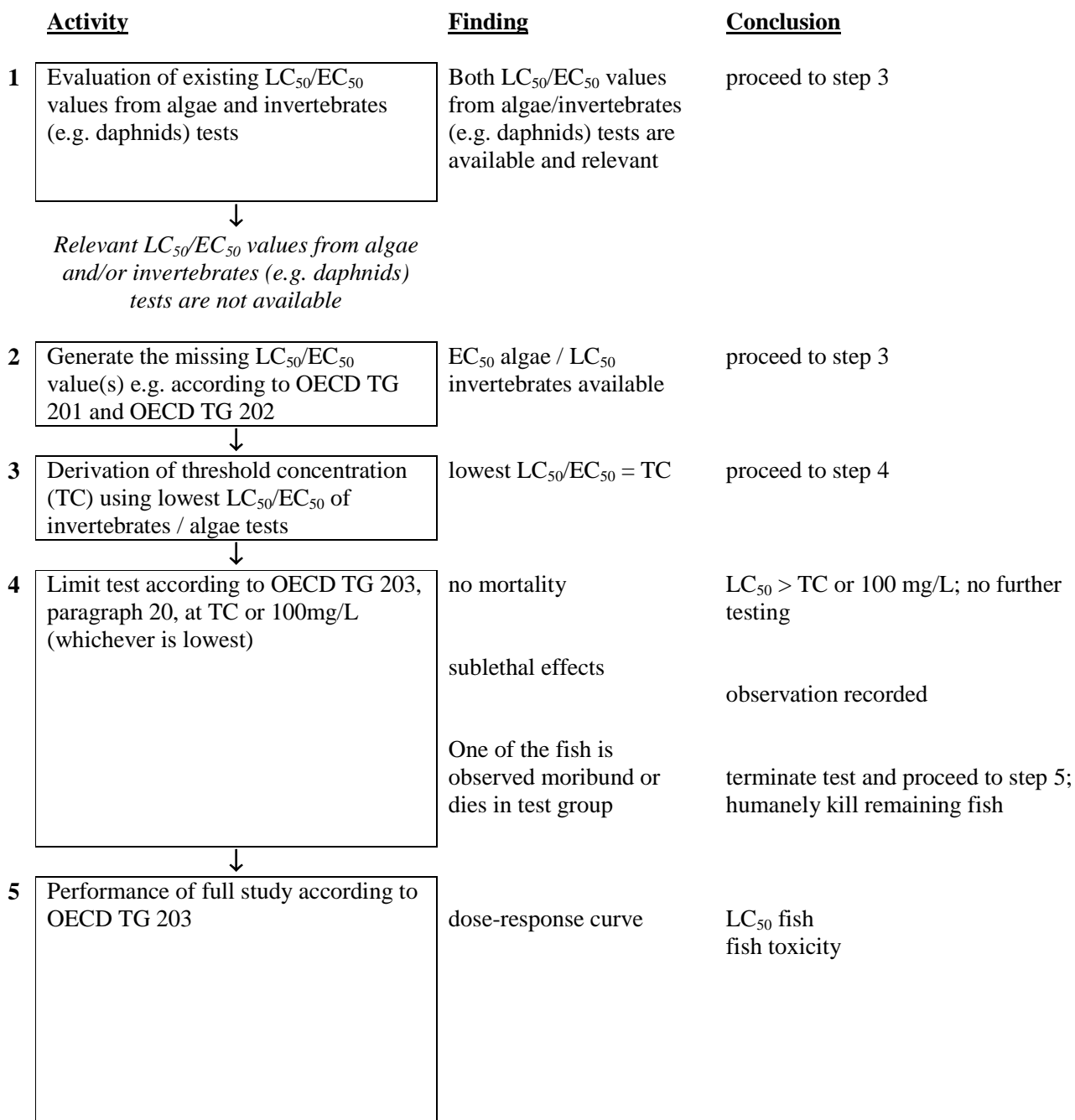
¹ Incorporated into the "Guidance on information requirements and chemical safety assessment" for REACH. See: ECHA (2008). Guidance on information requirements and chemical safety assessment. Chapter R.7B – Endpoint specific guidance (p. 41 ff, Chapter 7.8)

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5. Derivation of the threshold concentration (Step 1 - 3): The lowest EC50 value of existing and reliable algae or acute invertebrate (e.g. daphnia) toxicity data is set as threshold concentration (TC). If these data are not available they need to be determined according to OECD TG 201 and OECD TG 202 or any other standard test method generating reliable data.
6. Assessment of acute fish toxicity (limit test) at the TC (Step 4): An acute fish test is performed according to the limit test (OECD TG 203, paragraph 20) at the TC. If the TC is >100 mg/l, the test substance concentration should be 100 mg/l in the limit test. The absence of mortality indicates that the fish is not the most sensitive group of test organism after short-term exposure and that, with at least 99% of confidence, the LC50 is greater than the threshold concentration. If sublethal effects are observed, these should be recorded. The test should be terminated when 1 fish of the test group dies or is moribund, since this finding requires a full study (step 5). In compliance with the OECD Guidance Document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (8), the remaining fish should be humanely killed.
7. Performance of a full OECD TG 203 (Step 5): If any mortality or a moribund fish are observed in the limit test using the TC, a full OECD TG 203 study should be conducted.

Figure

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LITERATURE

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- (4) ECVAM (2006). Statement of the ECVAM Scientific Advisory Committee on the Scientific validity of the Upper Threshold Concentration (UTC) step-down approach for acute Aquatic Toxicity testing. ECVAM website: <http://ecvam.jrc.it/index.htm> (Validated Methods) and ATLA 35, 199-208, 2006.
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- (7) Sewell (2008). Reduction in the numbers of fish used in aquatic acute toxicity testing. Poster presentation at SETAC Europe 18th Annual Meeting 25-29 May 2008, abstract no MO 253.
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